A New Method of Detection of Pebrine Disease in Tasar Silk Moth, *Antheraea mylitta* Drury (Saturniidae)

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In the culture of *Antheraea mylitta* Drury, a semidomesticated Tasar Silk Moth, eggs of mother moths infected with *Nosema sp.*, (microsporidian) must be discarded to avert any catastrophe on crops caused by this pathogen. The infected mother moths (pebrine diseased) are detected by a method derived from that used in sericulture (Pasteur, 1870). In this method, the abdomen of an adult is severed with scissors, placed in a small mortar, mixed with water and crushed with pestle. A drop of the smear is placed on a clean slide and examined under a microscope for *Nosema sp.*, spores. This operation is most important but also time consuming in large grainages (insectaries where pupae of *A. mylitta* in their cocoons are held and at the onset of emergence of adults, eggs produced are processed). In the present study, technique is described to shift the time of microscopic examination by examining the exuviae, which remain in cocoon shells after pupation, instead of gut examination of mother moths. The new method and its advantages are discussed.

The exuviae used in this study were from diapausing pupae of *A. mylitta* (Fig. 1) reared during August-September, 1991 on primary host plants *Terminalia tomentosa* Wright and Arnon and *Terminalia arjuna* Bedd raised at the fields of the Central Tasar Research and Training Institute, Ranchi, India. As pebrine disease can be acquired from mother moths (primary infection) or from the environment through food (secondary infection), spores of *Nosema sp.* can be detected during any stage of the life cycle. Pupae selected for this study were of three types: those raised from eggs laid by (1) pebrine infected mothers, (2) pebrine-free mothers later inoculated with *Nosema sp.* spores during mid III instar and (3) pebrine free mothers (Control). 100 males and 100 females of each type, divided into five replications, were selected. Pupae were examined side by side with their exuviae to determine presence or absence of the disease.

The specimens for microscopic examination were processed in two ways viz., a) conventional and b) centrifuge methods:

a) conventional method: pupae were first washed with distilled water for two minutes, then the lower half of the abdomen (gut) was placed in a clean mortar. The tissue was crushed and the smear examined under microscope at 675 × magnification for *Nosema* spores.

b) Centrifugal method: The respective exuviae of the pupae were crushed with 5 ml of 2 % KOH in a mortar with pestle, let stand for 3

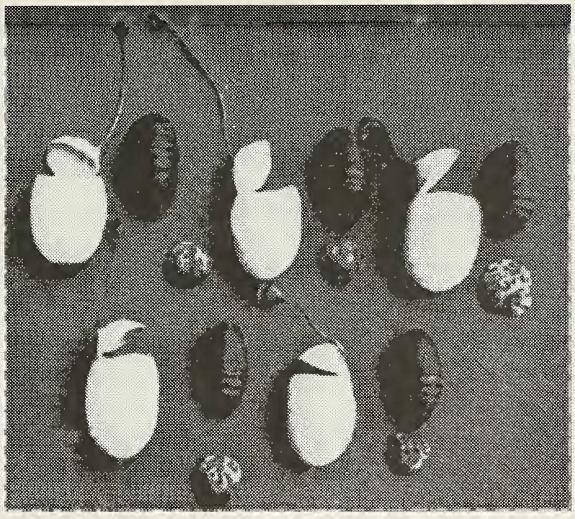

Fig. 1 Photograph of a pupa of *Antheraea mylitta* Drury showing its cocoon shell, pupa and exuviae.

minutes, mixed and filtered. The filterate was centrifuged at 500 rpm for 30 seconds. The supernatant was decanted and made up to 5 ml. with distilled water. The same was centrifuged at 2000 rpm for 10 minutes. The sediment was then smeared on a clean slide and five fields were examined for *Nosema* spores.

Results are illustrated in Table 1. *Nosema* spores were found in the body content as well as in their exuviae of the pupae, which became infected through their mother moths. There was no difference in the percentage of infection due to conventional or centrifugal methods or between sexes. Thus, instead of gut examination of mother moths, their exuviae may be examined to eliminate those individuals which acquired pebrine disease from their mothers.

In secondary infection, *Nosema sp*. spore-bearing pupae were higher in number than in exuviae. Observations made on external symptoms of pebrinised larvae of *A. mylitta* indicate that when I or early II instar larvae are inoculated with *Nosema* spores, black spots appear on the skin of larvae of III and early IV instars, but disappears in final instar (V). This indicates a relationship with detection of *Nosema* spores in exuviae in those individuals which acquired infection during their feeding stages. The individuals which were secondarily infected by *Nosema* during different stages of their larval life require detailed and systematic study with regard to: a) time required for appearance of black spots on the skin from the time of infection, b) examination of the molted skins for infection, c) intensity of infection in various organs and their route of migration to different tissues in larvae, pupae and adults, d) difference of infection between sexes, and e) mode of entry of *Nosema* spores into eggs. Only after these studies, pupae raised from larvae infected during

Table 1. Results of Microscopic Examination of pupae and their exuviae.

Sl. #	Type of Infection	Sex	% of pupae found Infected		% of exuivae found Infected		Remarks
			a	b	a	b	
1.	Primary	♂	100	100	100	100	Raised from infected mother moth
2.	Primary	♀	100	100	100	100	Raised from infected mother moth
3.	Secondary	♂	90	96	67	71	Inoculated with *Nosema* spores in mid III instar of larval stage
4.	Secondary	♀	92	95	53	67	Inoculated with *Nosema* spores in mid III instar of larval stage
5.	Control	♂	0	0	0	0	Infection free
6.	Control	♀	0	0	0	0	Infection free

Note: (a) = Conventional and (b) = Centrifugal methods of detection of infection.

feeding in the field may be screened for *Nosema* infection in *A. mylitta* by this method.

The present accepted method of pebrine detection in grainage is solely based on adults. This includes microscopic gut examination of mother moths for microsporidia spores (Pasteur, 1870), use of India ink in the microscopic field (Geetha Bai, et al., 1985) for dry moth testing, enzyme-linked immunosorbent assay, ELISA, (Kawarabata and Hayasaka, 1987), indirect fluorescent antibody techniques, (Sato, et al., 1981, Huang et al. 1983), latex bead agglutination, (Hayasaka and Ayuzawa, 1987), fluorescent antibody technique, (Huang, 1983), slide agglutination test (Hyasaka, 1983 and Li, 1985), and monoclonal antibody detection (Zhaoxi, et al., 1990). All these methods are accurate, but are cumbersome for large commercial grainages by requiring expensive laboratory facilities and skilled personnel. Pebrine detection through microscopic examination of exuviae may help the tasar industry to produce quality breeding material.

Tropical tasar silkworm diapausing pupae are preserved from November to May in bivoltine and February to May in trivoltine races. During this preservation period, exuviae examination for *Nosema* spore bearing insects can be done in the month of May. This reduces microscopic examination activities from production time. During production time, including moth eclosion, mating, oviposition and processing of eggs, the microscopic examination of mother moths must be done during a short span of 15 to 20 days for a stock of nearly 400,000 to 500,000 cocoons. This is laborious and time consuming, therefore affecting the quality of seed

production. The new method has an advantage of distributing the grainage work evenly from May to June instead of demanding all activities during June.

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